Is there any consistent structural and functional brain abnormality in narcolepsy? A meta-analytic perspective

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Narcolepsy is characterized by excessive daytime sleepiness, loss of muscle tone or cataplexy, sleep paralysis and hallucinations. Recently, several studies aimed to elucidate brain alterations in narcolepsy. However, their results are mostly inconsistent, demonstrating atrophy or disrupted activity/connectivity in various brain regions. This highlights the need for consolidating the neuroimaging literature on narcolepsy using meta-analytic approaches.

Accordingly, an activation likelihood estimation (ALE) meta-analysis investigated the spatial convergence of eight voxel-based morphometry (VBM) studies on narcolepsy and reported consistent atrophy in several regions including the hypothalamus, thalamus, basal ganglia, cingulate, middle orbital, inferior frontal, and superior temporal gyri (Weng et al., 2015). However, it was performed using GingerALE 2.1.1, which was later found to contain errors in implementation of false discovery rate (FDR) multiple comparisons correction. Subsequently, with the errors resolved in an updated version of GingerALE 2.3.3, the same data was re-analyzed by another team, who in contrast identified no significant convergence of the reported findings after FDR correction (Tanasescu et al., 2015). Zhong and colleagues re-performed meta-analysis using an alternative approach i.e., Seed-based d Mapping (SDM), arguing that unlike ALE it allows for including positive, negative and non-significant results in the same analysis, and can estimate heterogeneity and publication bias. They identified convergent atrophy of several regions i.e., the hypothalamus, striatum, thalamus, and superior and inferior frontal gyri, albeit using a highly lenient approach of multiple comparisons correction, i.e., an uncorrected voxel threshold of $p<0.001$ and a cluster extent of 10 voxels (Zhong et al., 2016). Therefore, and due to the insufficient control of the false positives in the previous meta-analyses, Tench and colleagues repeated the analysis on the same data, but using a stringent alternative for multiple comparisons correction, i.e., cluster-level family-wise error (cFWE), and observed significant convergence in the hippocampus. Of note, when they removed ROI-based coordinates of two experiments (which were mistakenly included in the previous meta-analyses) this finding vanished (Tench et al., 2019). Although this study carefully avoided repeating the methodological issues of the previous meta-analyses, they had similarly included only eight (seven without the ROI study) experiments. The small number of included experiments in a CBMA limits its sensitivity and increases the risk of an individual experiment dominating the results (Tahmasian et al., 2019).

Here, we aimed to extend the previous structural CBMAs on narcolepsy by performing a pre-registered ALE meta-analysis (PROSPERO, CRD42018105890) on both structural and functional neuroimaging experiments, while adhering to the best-practice guidelines for conducting CBMAs (Tahmasian et al., 2019). We searched PubMed, Web of Science and Embase in September 2020, performed reference tracking of relevant publications (Table S1), and after screening 2577 records, read 125 full papers and finally included 15 whole-brain neuroimaging experiments with 255 narcolepsy and 278 controls (Figure S1 ,Table S2). Next, we extracted the peak coordinates of the significant regions reported in the included

experiments and performed ALE meta-analyses (available at https://doi.org/10.6084/m9.figshare.16763179.v1). ALE treats the reported coordinates as spatial probability distributions, while assuming higher spatial certainty (i.e., with narrower distributions) for larger samples, and after combining these probability distributions into the ALE map, uses a permutation procedure to differentiate between true convergence and random clustering (Tahmasian et al., 2019). We used ALE as it properly accounts for the spatial uncertainty of the foci, is thoroughly evaluated as the most common CBMA method, and provides a stringent control for spurious findings in multiple comparisons (Tahmasian et al., 2019). We corrected for multiple comparisons using cFWE at $p<0.05$ and excluded coordinates from ROI-based experiments. Of note, we merged the data from multiple experiments performed on (partially) overlapping samples, whether reported in a single or multiple publications, to prevent them from overly influencing the results (Turkeltaub et al., 2012). Publications with overlapping samples were identified by examining their list of authors, location and time of the study, and sample demographics, although covert duplicates may still exist (Tramèr et al., 1997).

Our ALE analysis revealed no significant regional convergence across all the experiments [N=15] ($p_{cFWE}>0.326$), as well as across the subset of experiments reporting decreased grey matter volume, activity or connectivity [N=13] ($p_{cFWE}>0.215$). Modality-specific ALE analyses across the functional [N=9] ($p_{cFWE}>0.290$) and structural [N=7] ($p_{cFWE}>0.408$) experiments similarly revealed no significant convergence (Figure S2).

We observed the same non-significant finding using cFWE as (Tench et al., 2019), using a similar methodology but with increased number of experiments and including functional neuroimaging experiments. According to the best-practice guideline, we searched various databases, set strict selection criteria, removed ROI-based studies, merged experiments with overlapping samples, and used a stringent method of multiple comparisons correction i.e., cFWE, which might explain why we did not observe widespread regional convergence as reported in the earlier CBMAs (Weng et al., 2015; Zhong et al., 2016). It should be noted that although multimodal CBMAs are promising tools for identifying consistent findings across a large number of studies, this advantage comes at the price of introducing additional heterogeneity. The trade-off between the number and the homogeneity of the included experiments is an inherent limitation of meta-analytic approaches, which is settled according to the research question of interest and the amount of available data. Here, with the aim of identifying convergent structural and functional neuroimaging findings in narcolepsy, we performed a multimodal CBMA. Of note, we additionally performed modality-specific meta-analyses which similarly revealed no significant convergence. However, these analyses probably have a limited sensitivity for detecting convergent effects due to their small number of included experiments (Tahmasian et al., 2019), and this does not exclude the possibility of

identifying convergent modality-specific findings once more data is available and more sensitive ALE analyses on each modality are feasible.

In conclusion, despite an increased number of the included experiments compared with the previous meta-analyses on narcolepsy, adding functional experiments, and following the best-practice guideline in terms of in-/exclusion criteria and stringent statistical analysis, we found no significant regional convergence using cFWE. This may be attributed to (a) the sub-optimal sensitivity of our meta-analysis to detect the convergence, due to the small number of experiments and their heterogeneity, (b) the clinical variability (e.g., patients with or without cataplexy, duration of narcolepsy) of the included experiment, (c) their methodological and analytical flexibility, which in combination with a lack of pre-registration (14/15 experiments) enables selective reporting, or (d) a potentially high false positive rates in the individual studies due to their insufficient control for multiple comparisons (uncorrected in 9 experiments) and small sample sizes (median: 34). Therefore, we recommend future neuroimaging studies on narcolepsy to (1) study larger sample sizes, possibly through multi-center collaborations like ENIGMA-Sleep workgroup, to increase power and mitigate site-specific idiosyncrasies, (2) avoid questionable research practices by pre-registering their studies, transparent reporting, and providing open access to their code and data, (3) use standardize experimental, preprocessing, and analytical methods, and (4) minimize spurious findings by adequately controlling for multiple comparisons, motion and nuisance covariates.

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